

SEQUENCE LISTINGS

<110> Hanmi Pharm. Co., Ltd.

<120> EXPRESSION VECTOR FOR SECRETING ANTIBODY FRAGMENT USING E. COLI SIGNAL SEQUENCE AND METHOD FOR MASS-PRODUCING ANTIBODY FRAGMENT

<130> Q94300

<140> 10/576,068

<141> 2006-04-14

<150> KR1020030072216

<151> 2003-10-16

<150> PCT/KR04/02625

<151> 2004-10-14

<160> 36

<170> KopatentIn 1.71

<210> 1

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 1  
ggaaagcttc gatcgacat ccagatgacc cagtctccat cctccctgtc tgcatctgta 60  
ggggacagag tcacc 75

<210> 2

<211> 80

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 2  
tggttttgc tgataccagg ctaagtaatt tctgatgccg tgacttgccc gacaagtgtat 60  
ggtgactctg tccccctacag 80

<210> 3

<211> 80

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

```

<400>      3
cctggtatca gcaaaaacca gggaaagccc ctaagtcct gatctatgct gcatccactt      60
tgcaatcagg ggtccccatct                                         80

<210>      4
<211>      80
<212>      DNA
<213>      Artificial Sequence

<220>
<223>      gene fragment of light chain variable region

<400>      4
aggctgttagg ctgctgatgg tgagagtcaa atctgtccca gatccactgc cactgaaccg      60
agatgggacc cctgattgca                                         80

<210>      5
<211>      80
<212>      DNA
<213>      Artificial Sequence

<220>
<223>      gene fragment of light chain variable region

<400>      5
ccatcagcag cctacagcct gaagatgttg caacttatta ctgtcaaagg tataaccgtg      60
caccgtatac ttttggccag                                         80

<210>      6
<211>      41
<212>      DNA
<213>      Artificial Sequence

<220>
<223>      gene fragment of light chain variable region

<400>      6
tttgatttcc accttggtcc cctggccaaa agtatacggt g      41

<210>      7
<211>      75
<212>      DNA
<213>      Artificial Sequence

<220>
<223>      gene fragment of heavy chain variable region

```

```

<400>    7
ggaaagcttc gatcgagggt gcagctggtg gagtctgggg gaggcttggg acagccggc          60
aggcccctga gactc                                         75

<210>    8
<211>    79
<212>    DNA
<213>    Artificial Sequence

<220>
<223>    gene fragment of heavy chain variable region

<400>    8
agcttgccgg acccagtgca tggcataatc atcaaaggtg aatccagagg ccgcacagga          60
gagtctcagg gacctgccg                                         79

<210>    9
<211>    80
<212>    DNA
<213>    Artificial Sequence

<220>
<223>    gene fragment of heavy chain variable region

<400>    9
tgcactgggt ccggcaagct ccagggaaagg gcctggaatg ggtctcagct atcacttgga          60
atagtggtca catagactat                                         80

<210>    10
<211>    80
<212>    DNA
<213>    Artificial Sequence

<220>
<223>    gene fragment of heavy chain variable region

<400>    10
atacagggag ttcttggcgt tgtctctgga gatggtaat cggccctcca cagagtccgc          60
atagtctatg tgaccactat                                         80

<210>    11
<211>    80
<212>    DNA
<213>    Artificial Sequence

<220>
<223>    gene fragment of heavy chain variable region

```

```

<400>    11
acgccaagaa ctccctgtat ctgcaa atga acagtctgag agctgaggat acggccgtat      60
attactgtgc gaaa gtctcg                                         80

<210>    12
<211>    84
<212>    DNA
<213>    Artificial Sequence

<220>
<223>    gene fragment of heavy chain variable region

<400>    12
cactcgagac ggtgaccagg gtaccttggc cccaatagtc aaggaggac gcggtgctaa      60
ggta cgagac tttcgac aca g taat                                         84

<210>    13
<211>    39
<212>    DNA
<213>    Artificial Sequence

<220>
<223>    RT-PCR forward primer specific for heavy chain

<400>    13
cccaagctta ggccctccacc aagggcccatt cggtcttcc                           39

<210>    14
<211>    48
<212>    DNA
<213>    Artificial Sequence

<220>
<223>    RT-PCR reverse primer specific for heavy chain

<400>    14
gggggatcct tatgggcacg gtgggcatgt gtgagtttg tcacaaga                  48

<210>    15
<211>    42
<212>    DNA
<213>    Artificial Sequence

<220>
<223>    RT-PCR forward primer specific for light chain

<400>    15

```

cccaagcttt	cgcgaactgt	ggctgcacca	tctgtcttca	tc	42	
<210>	16					
<211>	42					
<212>	DNA					
<213>	Artificial Sequence					
<220>						
<223>	RT-PCR reverse primer specific for light chain					
<400>	16					
cccgatccc	taacactctc	ccctgttgaa	gctctttgtg	ac	42	
<210>	17					
<211>	69					
<212>	DNA					
<213>	modified E. coli thermostable enterotoxin II signal sequence					
<400>	17					
atgaaaaaga	caatcgatt	tcttcttgca	tctatgttcg	ttttttctat	tgctacaaat	60
gcccaggcgc						69
<210>	18					
<211>	45					
<212>	DNA					
<213>	Artificial Sequence					
<220>						
<223>	forward primer containing StuI restriction enzyme site					
<400>	18					
tctattgcta	caaattcccc	ggccttcccc	accattccct	tatcc	45	
<210>	19					
<211>	45					
<212>	DNA					
<213>	Artificial Sequence					
<220>						
<223>	reverse primer containing StuI restriction enzyme site					
<400>	19					
agataaacgt	gtttacgggt	ccggaagggt	tggtaaggga	atagg	45	
<210>	20					
<211>	51					
<212>	DNA					
<213>	Artificial Sequence					
<220>						

<223> reverse primer specific for light chain

<400> 20  
ggggatcct cacgcggcgc atgtgtgagt tttgtcacaa gattnaggct c 51

<210> 21  
<211> 43  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing SD sequence and BamHI restriction enzyme site

<400> 21  
ggggatcca ggaggtgatt tatgaaaaag acaatcgcat ttc 43

<210> 22  
<211> 44  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing BpuI restriction enzyme site

<400> 22  
ggggctgagc aggaggtgat ttatgaaaaa gacaatcgca ttcc 44

<210> 23  
<211> 52  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing BpuI restriction enzyme site

<400> 23  
ggggctcagc tcacgcggcgc catgtgtgag ttttgtcaca agattnaggc tc 52

<210> 24  
<211> 63  
<212> DNA  
<213> E. coli OmpA signal sequence

<400> 24  
atgaaaaaga cagctatcgc gattgcagtgc actggctg gtttcgtac cgttgcgc 60  
gct 63

<210> 25  
<211> 30

<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer specific for heavy chain

<400> 25  
gaggttcagc tagtcgagtc aggaggcggt 30

<210> 26  
<211> 51  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing HindIII and StuI restriction enzyme sites

<400> 26  
gggagatctt cacgcggcgc atgtgtgagt tttgtcacaa gatttaggct c 51

<210> 27  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 27  
gacattcaaaa tgacccagag cccatccagc 30

<210> 28  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing HindIII and NruI restriction enzyme sites

<400> 28  
cccagatctc taacactctc ccctgttgaa gctcttggc ac 42

<210> 29  
<211> 41  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 29		
ggggtcgaca ggaggtgatt tatgaaaaag acagctatcg c		41
<210> 30		
<211> 51		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> reverse primer containing SalI restriction enzyme site		
<400> 30		
ggggtcgact cacgcggcgc atgtgtgagt tttgtcacaa gatttaggct c		51
<210> 31		
<211> 42		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> forward primer specific for modified E. coli enterotoxin II signal peptide and containing NdeI restriction enzyme site		
<400> 31		
gggcatatga aaaagacaat cgcatttctt cttgcacatcta tg		42
<210> 32		
<211> 705		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> TNF-alpha heavy chain		
<400> 32		
gaggttcagc tagtcagtc aggaggcggt ttggtaacgc ccggcaggc cctgagactc		60
tcctgtgcgg cctctggatt caccttgat gattatgcc a tgcactgggt ccggcaagct		120
ccagggagg gcctggaatg ggtctcagct atcacttgg a atagtggtca catagactat		180
gcggactctg tggagggccg attcaccatc tccagagaca acgccaagaa ctccctgtat		240
ctgcaa atga acagtctgag agctgaggat acggccgtat attactgtgc gaaagtctcg		300
taccttagca ccgcgtcctc ccttgactat tggggccaag gtaccctggt caccgtctcg		360
agtgcctcca ccaaggccc atcggcttcc cccctggcac cctcctccaa gagcacctct		420
gggggcacag cggccctggg ctgcctggc aaggactact tccccgaacc ggtgacggtg		480
tcgtgaaact caggcgccct gaccagcgcc gtgcacacct tcccggtgt cctacagtcc		540

tcaggactct actccctcag cagcgtggtg accgtgcctt ccagcagctt gggcacccag 600  
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagtttag 660  
cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc catag 705

<210> 33  
<211> 645  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> TNF-alpha light chain

<400> 33  
gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgttagggga cagagtcacc 60  
atcacttgtc gggcaagtca gggcatcaga aattacttag cctggtatca gaaaaacca 120  
gggaaagccc ctaagctcct gatctatgct gcattcactt tgcaatcagg ggtcccatct 180  
cggttcagtg gcagtggatc tggacagat ttcactctca ccatcagcag cctacagcct 240  
gaagatgttgc caacttatta ctgtcaaagg tataaccgtg caccgtatac ttttggccag 300  
gggaccaagg tggaaatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca 360  
tctgatgagc agttgaaatc tggaaactgcc tctgttgtgt gcctgctgaa taacttctat 420  
cccagagagg ccaaagtaca gtggaaagggtg gataacgccc tccaatcggg taactcccag 480  
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540  
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcaggc 600  
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag 645

<210> 34  
<211> 7  
<212> PRT  
<213> TNF-alpha light chain

<400> 34  
Asp Ile Gln Met Thr Gln Ser  
1 5

<210> 35  
<211> 8  
<212> PRT  
<213> TNF-alpha heavy chain

<400> 35  
Glu Val Gln Leu Glu Val Asp Ser  
1 5

<210> 36  
<211> 12  
<212> PRT  
<213> N-terminal sequence of recombinant TNF-alpha

<400> 36  
Asp Glu Ile Val Gln Met Leu Thr Val Gln Asp Ser  
1 5 10